REMARKS

Favorable reconsideration of this application, in light of the preceding amendments and following remarks, is respectfully requested.

Claim 46 is amended to recite a process for producing a plant culture cell for protein production including a first transforming step of transfecting a plant culture cell with a plasmid comprising a transcription factor-expressing DNA fragment comprising a coding gene of a transcription factor and a promoter for expressing the transcription factor; a screening step of screening plant culture cells, obtained in the first transforming step, for an individual plant culture cell expressing the transcription factor; and a second transforming step of transfecting the individual plant culture cell, obtained in the screening step, with a plasmid comprising a protein-expressing DNA fragment comprising cDNA of a RNA virus vector, having incorporated therein a gene encoding a target protein into the RNA virus vector cDNA; an inducible promoter which is induced by the transcription factor; and a ribozyme sequence of satellite tobacco ringspot virus ligated to the 3' end of the RNA virus vector cDNA. Support for such amendment is found in at least Claims 46, 55 and 56 as originally filed.

Minor amendments are made to Claims 47, 48, and 50-54 for clarity, to correct grammar, and/or fix antecedent basis.

Claim 57 is amended for clarity purposes to a process for producing a plant culture cell for protein production as set forth in Claim 46, wherein the gene encoding the target protein replaces a viral coat protein gene. Support for Claim 57 is found in the specification as filed at least at originally-filed Claim 57.

No new matter is added, and no change of inventorship is believed to result from the amendment of claims as proposed herein.

Applicant reserves the right to reintroduce any cancelled subject matter in one or more later-filed continuation applications.

RESPONSE TO OFFICE ACTION DATE 31 MAY 2011

1. Claim Objection

The Office Action (p. 1) objects to Claim 46 and states "the metes and bounds of the claim are clear in light of the teachings of the specification, [however,] the wording of the phrase 'a protein-expressing DNA fragment in which cDNA of a virus vector that has been constructed by inserting a coding gene of an arbitrary protein into an RNA virus is ligated to an inducible promoter which is induced by the transcription factor' is confusing."

Applicant has amended Claim 46 herein to further enhance clarity and has added commas as suggested by the Examiner. Therefore, Applicant respectfully requests withdrawal of the Claim 46 objection.

2. 35 U.S.C. § 112, second paragraph

Claims 57 and 64 stand rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. This rejection is respectfully traversed.

The Office Action (p. 2) states "it would be remedial to amend the claim to recite 'wherein the coding gene of the arbitrary protein replaces a coat protein gene so that there will be no production of the viral coat protein.'" Claim 57 has been amended to recite the gene encoding the target protein replaces a viral coat protein gene. Therefore, the "metes and bounds" of Claim 57 is clear.

Claim 64 has been cancelled and thus, such rejection is moot.

Applicant respectfully requests withdrawal of the 35 U.S.C. §112, second paragraph rejection of Claims 57 and 64.

3. 35 U.S.C. § 112, fourth paragraph

Claims 57 and 64 stand rejected under 35 U.S.C. §112, fourth paragraph as allegedly being improper dependent form for failing to further limit the subject matter of the previous claim. This rejection is respectfully traversed, however, Claim 57 is amended herein, and therefore this rejection is moot. Claim 64 has been cancelled and thus, such rejection is moot.

Applicant respectfully requests withdrawal of the 35 U.S.C. §112, second paragraph rejection of Claims 57 and 64.

4. Rejections under 35 U.S.C. § 103

A. Garger in view of Weber and Zuo

Claims 24, 31, 37, 39, and 40 stand rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over U.S. Patent Publication No. 2002/0061309 (hereinafter Garger) in view of Weber, *et al.* Journal of Virology, 66:6, p. 3909-3912 (June 1992) (hereinafter Weber) and U.S. Patent No. 6,452, 068 (hereinafter Zuo). Applicant respectfully traverses this rejection, however, in view of the current amendments, such rejection is moot as Claims 24, 31, 37, 39, and 40 are cancelled herein.

Applicant respectfully requests withdrawal of the 35 U.S.C. §103 rejection of Claims 24, 31, 37, 39, and 40.

B. Mori in view of David

Claims 46-48, 50, 51, 56-58 and 60-64 stand rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Mori *et al.*, The Plant Journal, 27:1, pages 79-86 (2001) (hereinafter Mori) in view of David *et al.*, Plant Physiology, Vol. 125, pages 1548-1553 (April 2001) (hereinafter David). Applicant respectfully traverses this rejection for the reasons detailed below.

Presently amended Claim 46 recites a process for producing a plant culture cell for protein production, comprising:

> a first transforming step of transfecting a plant culture cell with a plasmid, the plasmid comprising a transcription factor-expressing DNA fragment comprising a coding gene of a transcription factor; and

a promoter for expressing the transcription factor;
a screening step of screening plant culture cells, obtained in the first transforming step, for an individual plant culture cell expressing the transcription factor; and
a **second transforming step** of transfecting the individual plant culture cell, obtained in the screening step, with a plasmid, **the plasmid comprising** a protein-expressing DNA fragment comprising
cDNA of a RNA virus vector, having incorporated therein a gene encoding a target protein into the RNA virus vector cDNA;
an inducible promoter which is induced by the transcription factor; and
a **ribozyme sequence** of satellite tobacco ringspot virus **ligated to the 3' end of the RNA virus vector cDNA**.

The Office Action (p. 24) concludes stating "[c]ombining the teachings of Mori et al and David et al results in a simple substitution of vectors used in the method of David et al with the vectors taught by Mori et al in order to obtain predictable results." The asserted conclusion is not supported by the evidence of record and thus, the Office Action fails to establish a presumption of *prima facie* obviousness for Claim 46 for the following reasons.

i. No Guidance to Modify the Alleged Combination of Documents

At the outset, Applicant respectfully submits that the Examiner does not provide a clear rationale why one of ordinary skill in the art would have been motivated to make and modify the proposed combination of Mori and David to arrive at Claim 46. A rationale is proposed, however the rationale does not address how one would (1) combine and (2) modify Mori and David to arrive at the process of Claim 46 as a whole. Instead, the Examiner makes the conclusory statement that it would have been obvious to one skilled in the art to combine Mori and David, given the expected benefit of selecting BY2GVG transformants with desirable levels of GVG expression prior to transformation with the IFN expression vector, and that BY2 cells have an exceptionally high growth rate and are easy to transform. *See* Office Action, p. 12. However, this does not address why one would be motivated to modify the alleged combination to include all of the elements of Claim 46 as a whole, especially why one would arrive at a second transformation step using a plasmid containing RNA virus vector cDNA. Neither Mori nor David mention RNA virus vector cDNA and there is no rationale presented for modifying the alleged combination to include this feature. Mori does transfect a whole plant with viral replicase. However, this is a completely different technical concept from the transfection of

plant culture cells with a plasmid containing RNA viral vector cDNA. Unlike the present invention, no viral vector cDNA is expressed in Mori and thereby no viral growth is caused.

Lastly, as previously articulated (Applicant's RCE dated 27 September 2010, p. 8), a normal plasmid (containing no viral cDNA) and a plasmid containing viral cDNA (a virus vector) are different from each other in terms of efficient expression, *i.e.* a virus vector tends to cause a deterioration in an expression efficiency. It was Applicant who discovered that a second transforming step involving a virus vector would not reduce expression efficiency, contrary to wisdom at the time of the invention. Accordingly, the Office's suggested combination of Mori and David and modification to David are contrary to one of ordinary skill's knowledge, at the time of invention, regarding virus vectors. Furthermore, the Office provides no explanation of how an additional transforming step with a virus vector (not disclosed in David) may be implemented in the process of Mori. Accordingly, there is no motivation for one of ordinary skill in the art to combine Mori and David and modify David as suggested by the Office.

ii. **The Cited Documents Fail To Disclose, Teach or Suggest Claimed Features**

Claim 46 recites a two-step plant culture cell transformation process. First, plant culture cells are transfected with a plasmid having the transcription factor-expressing DNA fragment. Then the plant culture cells are screened for expressing the transcription factor. Thereafter, the individual plant culture cells which are positive for expressing the transcription factor are transfected with a plasmid having DNA to which the following (i) and (ii) are ligated: (i) RNA virus vector cDNA in which a gene encoding the expression-target protein is inserted and a ribozyme sequence of satellite tobacco ringspot ligated to the 3' end of the RNA virus vector cDNA and (ii) an inducible promoter which is induced by the transcription factor from the first transformation step. This causes the expression of the RNA virus vector cDNA in which the gene encoding the expression-target protein is inserted. Transcription of the RNA virus vector cDNA is induced by the transcription factor, so that the gene encoding the expression-target protein in the RNA virus vector cDNA is expressed. Thus, the expression-target protein in the individual plant culture cell is produced by use of the RNA virus vector cDNA from the plasmid.

Mori and David (alone or in combination) do not teach or suggest at least the following two claim elements:

1. **RNA virus vector cDNA:** Neither Mori nor David discloses, teaches or suggests transforming a plant culture cell first to express a transcription factor, and after screening, transforming the plant culture cell again with a plasmid containing cDNA of a RNA virus vector and the target protein, wherein the plasmid is induced by the transcription factor expressed in the first step. The Office Action (p. 11) admits that "Mori et al do not teach the method where the GVG transformants are further transformed with the cDNA of a virus vector that encodes IFN using an Agrobacterium method." Instead, Mori transfects a whole plant with virus replicase. However, unlike the present Application, no virus vector is expressed and thereby no viral growth is caused in Mori. Further, this deficiency is not cured by David. Although David does report a serial transformation in BY2 cells, David is silent regarding a second transformation with a plasmid containing RNA virus vector cDNA and also having an inducible promoter controlled by the transcription factor expressed from the first transformation.

2. **Plasmid Arrangement:** Further, neither Mori nor David discloses, teaches or suggests a process in which a ribozyme sequence is ligated to the 3' end of the RNA virus vector cDNA. Contrary to the Office Action, Mori has a ribozyme sequence ligated to the 3'end of the target gene, IFN, not the viral cDNA. Accordingly, with Mori and David failing to disclose a further element of the process, the combination of Mori and David fail to disclose all of the elements of Claim 46.

iii. **No Reasonable Expectation of Success**

Even if motivation existed to combine or modify the cited documents (which is not admitted herein), as paraphrased in MPEP 2143.01(III), "[t]he mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art." Since the cited references, Mori and David, do not disclose a two-step transformation wherein the second transformation transfects a plant culture cell with cDNA of a RNA virus vector or a ribozyme sequence ligated to the 3' end of the RNA virus vector cDNA, one of ordinary skill in the art could not predict the effectiveness of this claimed process using RNA virus vector cDNA for at least the following reasons.

1. Viral vector cDNA is difficult and unpredictable:

Transformation of a plant by use of a virus vector cDNA is difficult and even more so for a plant culture cell. That is, even in a case where the plant is transfected with multiple genes by using a single virus vector, expression efficiency of the multiple genes is very low. Such a problem does not arise if no virus vector is employed, *i.e.*, if a vector other than a virus vector, e.g., plasmid containing no viral DNA, is employed. In other words, the problem arises selectively in a case where a virus vector is employed. The problems are more serious in a case where a plant culture cell is transformed than in a case where a plant is transformed. All leading one of ordinary skill in the art to have an expectation of **failure** in the claimed process, involving a two-transformation-step process for producing a plant culture cell for protein production with the second transforming step including cDNA of a RNA virus vector and a ribozyme sequence ligated to the 3' end of the RNA virus vector cDNA.

By way of example, in a case where a plant culture cell is transfected with (i) a GFP gene and (ii) a transcription factor for transcription induction by using a single plasmid, an expression efficiency (a ratio of cells which emit light) to be obtained is approximately 100%. Also, in a case where a plant is transfected with (i) and (ii) by using a single plasmid, expression efficiency to be obtained is approximately 100 %. On the other hand, in, a case where a plant is transfected with (i) and (ii) by using a single virus vector, expression efficiency to be obtained is approximately 30 % at maximum. Expression efficiency is further lowered to be less than 5 % in a case where a plant culture cell is transfected with (i) and (ii) by using a single virus vector (see the result of the use of the control vector in Example 2 in the specification as filed).

2. Viral growth at the growing point is difficult in plant culture cells vs. plants:

It is also known by one of ordinary skill in the art that viral growth at the growing point is difficult. In the plant, a virus can migrate between cells to spread throughout the entire plant, whereas such viral migration and spreading does not occur in plant culture cells. As understood from this, it is very difficult to cause viral growths in all plant culture cells. No conventional plant techniques are directly applicable to overcome such difficulty. Additionally, plant culture cells in which no virus growth occurs multiply at faster rates than plant culture cells in which virus growth does occur. In view of this, it is necessary to cause virus growth in every plant

culture cell. However, since no virus can migrate between plant culture cells, to cause the virus growth in every plant culture cell imposes a previously unsolved technical problem. Again, all leading one of ordinary skill in the art to have an expectation of **failure** in the claimed process.

3. **No conventional protoplast formation needed with claimed features**

As discussed more fully above in Sec. ii, both Mori and David are silent on the ribozyme sequence being ligated to the 3' end of the RNA viral vector cDNA. In order for conventional techniques to express protein in plant culture cells, it is necessary that the plant culture cells be formed into protoplasts. For example, this is also true in Mori and David. In contrast, Applicant discovered that unpredictably the claimed features of (1) the arrangement of the ribozyme of the satellite tobacco ringspot virus ligated to the 3' end of the RNA viral vector cDNA and (2) the two-step transformation caused protein expression in plant culture cells without the formation of protoplasts.

4. **Viral vector cDNA expression is low in plant culture cells**

Protein production in plant culture cells is different from protein production in plants. Use of plant culture cells is much more unpredictable and this is generally known in the art. For example, in a case where chromosomes of the plant culture cells are transfected with virus vector genes to form transformant culture cells, virus vectors expressed in almost all of the transformant culture cells are chimerical. As such, probability that the virus vectors are expressed as intended in the transformant culture cells is only a few % at maximum.

Therefore, it was Applicant who first discovered that use of two transforming-steps using RNA virus vector cDNA and a ribozyme sequence ligated to the 3' end of the RNA virus vector cDNA could effectively produce a plant culture cell with improved protein expression. Because one of ordinary skill would expect failure in using plant culture cells and failure in use of virus vector cDNA (for the multiple reasons articulated above), it was unpredictable that Applicant's claimed process improved expression efficiency. As set forth in Example 2 (p. 64-65 of the specification as filed), the percentage of GFP-expressing cells was unpredictably about 60% in the vector containing the ribozyme sequence of satellite tobacco ringspot virus. The Office Action (p. 17) implies Example 2 involves a plasmid vector and not a virus vector. However, the Office Action (p. 18, emphasis added) also states "the results of the experiment demonstrate that

the ribozyme sequence of satellite tobacco ringspot virus provides higher levels of expression in BY2 cells when the ribozyme is convalently bonded to the 3' end of a tomato mosaic **virus** cDNA." Accordingly, the Office recognizes Example 2 does exemplify the unpredictable efficiency of using a **virus vector cDNA** in a plasmid for the expression of GFP. Further, the Office Action (p. 18, emphasis added) concludes that "Example 2 is not a working example of the claimed invention, and the claims are not commensurate in scope with any unexpected improvement in expression efficiency that may **have been obtained using the ribozyme sequence of satellite tobacco ringspot virus** in the particular system used in Example 2." However, amended Claim 46 includes a ribozyme sequence of satellite tobacco ringspot virus ligated to the 3' end of the RNA virus vector cDNA. Accordingly, Example 2 exemplifies the unpredictable effectiveness of using a virus vector containing the ribozyme sequence of satellite tobacco ringspot virus ligated to the 3'end of the RNA virus vector cDNA.

Furthermore, Applicant's Example 3 is a two-transforming step process, which unpredictably resulted in improved efficiency. In response to Applicant's Example 3 data showing unpredictability, the Office Action (p. 18) states "[i]t is unclear how the stable expression of a protein in the plant cell of Example 3 differs from that of the prior art." Since neither a second transforming step using RNA virus vector cDNA nor a ribozyme sequence ligated to the 3' end of the RNA virus vector cDNA are disclosed, taught or suggested in the cited art, the stable expression of protein via the Example 3 process is both different from that of the cited art and unpredictable in light of the expectation of failure (as articulated above) in the art at the time of the invention. According, Applicant respectfully submits that the claimed process was unpredictable at the time of invention.

Claims 47-48, 50, 51, 57, 58, and 60-63, dependent on independent claim 46, are patentable for the reasons stated above with respect to claim 46 as well as for their own merits. Claim 56 has been cancelled, and thus, such rejection is moot.

Applicant respectfully requests withdrawal of the 35 U.S.C. §103 rejection.

C. Mori in view of David and Zuo

Claim 49 stands rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Mori in view of David and Zuo. Applicant respectfully traverses this rejection for the reasons detailed below.

Even assuming *arguendo* that David and Zuo could be combined with Mori (which Applicants do not admit), the Examiner has failed to show how David and Zuo remedy the deficiencies of Mori with respect to independent Claim 46. Thus, Claim 49, dependent on independent claim 46, is patentable over Mori, David and Zuo for at least the reasons set forth above with respect to independent Claim 46.

Applicant, therefore, respectfully requests that the rejection to Claim 49 under 35 U.S.C. § 103(a) be withdrawn.

D. Mori in view of David and Rasochova

Claims 52-54 stand rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Mori in view of David and further in view of U.S. Patent Publication No. 2003/0074677 (hereinafter Rasochova).

Even assuming *arguendo* that David and Rasochova could be combined with Mori (which Applicant does not admit), the Examiner has failed to show how David and Rasochova remedy the deficiencies of Mori with respect to independent Claim 46. Thus, Claims 52-54, ultimately dependent on independent Claim 46, is patentable over Mori, David and Rasochova for at least the reasons set forth above with respect to independent Claim 46.

The Applicants, therefore, respectfully request that the rejection to Claims 52-54 under 35 U.S.C. § 103(a) be withdrawn.

5. Conclusion

Accordingly, in view of the above amendments and remarks, reconsideration of the objections and rejections and allowance of each of claims in connection with the present application is earnestly solicited.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Donald J. Daley at the telephone number of the undersigned below.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. **08-0750** for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. §1.17; particularly, extension of time fees.

Respectfully submitted,

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